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VANADIUM IN CHARCOT-MARIE MUSCULAR ATROPHY
[Vanadio no Amiotrofia de Charcot-Marie]

by

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Four patients with Charcot-Marie amyotrophy were studied concerning vanadium metabolism. The method of Talvitie was used to determine the pressure of vanadium in blood serum. The metabolism of vanadium is discussed in relation to the amyotrophy. The patients present low values of vanadium in blood serum which are proportional to the severety of the disease.

Introduction

Charcot-Marie is a type of progressive muscular atrophy, of a family and hereditary nature, which begins in infancy or in adolescence. The atrophy reaches the muscles of the anteroexternal region of the legs and later on the muscles of the hands and of the forearms. It results in a talipes and pes cavus,

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in a garter atrophy of the lower members and a Aran-Duchenne type hand. This type of amyotrophy includes degenerative lesions of the posterior cords of the medulla, in the cells of the anterior horns and in the peripheral nerves (Litter and Wexselblatt, 1944; van Bogaert, 1949), [18, 40].

The chemical element vanadium, s^2d^3, presents biological analogy with iron (s^2d^6) and copper s^2d^9 or s^2d^{10}), and here we observe a sequence 3,6,9, applicable to the number of electrons in the energy sublevel d (De Jorge, et al., 1966) [12].

Animals of the Tunicata family have appreciable quantities of this metal, a fact which was discovered by Henze (1911) [14] when he studied the blood of the Phallusia mamillata species. Later investigations gave evidence of the presence of a chromcprotein, hemovanadine, containing vanadium in a trivalent form bonded to the sulfate anion and with reduction properties (Henze, 1912, 1913; Henze et al., 1932; Webb, 1939; Califano and Boeri, 1950; Boeri and Ehrenberg, 1953; Bielig and Bayer, 1954) [15, 16, 17, 41, 6, 5, 4].

Research performed by Noddack and Noddack (1940) [28] as well as that of Bertrand (1950) [3] revealed the presence of vanadium in the tissues of animals; studies on humans were made by Koch et al (1956) [17a], Perry and Perry (1959) [29] and Tripton (1960) [38], and the accumulation of vanadium in the lung is interesting to observe.

By virtue of the interest in the investigations of amyotrophies (van Bogaert, 1949) [40] as well as in the study of the metabolism of vanadium (Faulkner Hudson, 1964) [13], we determined the concentrations of this metal in the blood serum of four patients of the same family with charcot-Marie amyotrophy and we compared the results with those of four individuals without any nervous or muscular alterations.

Patients

Case 1 -- age 12, female, complaining of pain in the upper members for two years. A neurological examination revealed a universal hyper reflex, with a discreet hypotony in the distal extremities of the members and a descreet motor deficit of the extremities. The psychism is maintained, sensitivity within normal bounds, and so are the cranial nerves. There is a discreet talipes and pes cavus , garter atrophy and an Aran-Duchenne type hand. A biopsy of the muscle and nerve revealed a muscular atrophy with partial hyalinization of the fibers, and an atrophy of the nerves, with a partial degeneration of the fibrils. A hemogram showed a reduction of the monocytes (1%) and an increase of the eosinophiles (24%). An examination of the feces showed the presence of ancillostomiasis eggs and of Ascaris lumbricoides. The following examinations were within normal bounds: cephalorachidian liquid, encephalogram, urine type I, and proteins, inorganic phosphor, potassium, sodium, calcium, chloride, urea, and alkaline reserve of the blood serum. An electromyographic examination with its traces compatible with spinal amyotrophy of chronic evolution.

Case 2 -- age 14, female, with pain in the lower members being felt for one year, progressive weakness hindering habitual movements; after some time, the pain also appeared in the upper members. A neurological examination revealed universal lack of reflexes, hypotonia in the distal extremities of the members with trophic alterations similar to those of Case 1. A histological examination of the biopsy material of the muscle and of the nerve presented accentuated muscular atrophy, with partial necrosis and a substitution by a fatty interstitial tissue. Hemogram with 16% of the eosinophiles and 1% of monocytes. The presence of ancillostomiasis eggs in the feces. The other examinations yielded results similar to those found in Case 1.

Case 3 -- age 23, male, symptoms beginning seven years ago in the upper mem-

bers and later on in the lower members, with progressive weakness until reaching incapacity to walk. A neurological examination revealed an accentuated hypotonia in the distal extremities of the members, with trophic alterations (cyanosis, scaling of the skin and fallout of hair), and a motor deficit in the extremities with the absence of voluntary movement of the feet. A histological examination of the biopsy of the muscle and nerve showing a nerve with its structure highly altered by atrophy of the fibers and the interstitial fibrosis, muscular atrophy with a fatty interstitial substitution. Other laboratory examinations with similar results to those encountered in the two previous cases.

Case 4 -- age 25, male, with beginning of the symptomatology dating back nine years. Neurological and laboratory test pattern similar to the previous one. The four brothers presented more or less three phases of the ailment's progress.

Analytical Method of Vanadium

Vanadium was determined by the method described by Talvitie (1953) [37a], with some modifications adapted for blood serum and for the soft tissues in general.

Reagents -- (1) 0.1% methyl orange indicator (dissolve 100 mg of methyl orange in 100 mg of distilled water); (2) 4 N nitric acid solution; (3) 4 N sulfuric acid solution; (4) 4 N ammonium hydroxide solution; (5) solution of ammoniacal buffer with pH = 9.4 (dissolve 200 ml of a 4 N solution of ammonium hydroxide and 100 ml of a 4 N solution of nitric acid in 2 l of distilled water); (6) a buffer solution of potassium phtalate, pH = 4.0 (dissolve 12/77 g of potassium biphtalate in water and dilute to 250 ml); (7) alcohol free chloroform (extract the alcohol of 2 l chloroform, R.A., shake vigorously with 6 portions precipitated out of 500 ml of distilled water each, and finally leave a small layer of water covering the chloroform and preserve in refrigerator); (8) 0.5% solution of 8-hydroxiquinoline (dissolve 5 g of 8-hydroxiquinoline in one liter of alcohol free chloroform); (9) 0.1% solution of 8-hydroxiquinoline; (10) standard solution of vanadium,

1 ml = 1 µg of vanadium (dissolve 0.296 g of ammonium metavanadate in 25 ml of 4 N solution of sulfuric acid and dilute for one liter with distilled water, preparing various dilute solutions, each 1 µg to 50 µg of vanadium per 1 ml.

Analytical method -- (1) Place 5 ml of blood serum in a crucible or a porcelain capsule, add 2 ml of concentrated nitric acid, digest on the heating plate. Care must be taken not to spill and not to have any projection of the material during that phase. This is achieved by agitating with a glass stick and withdrawing the crucible from the heating plate as the material increases in volume. (2) After the material has been completely digested and dried, it is recommended to place the crucible inside the furnace and to raise the temperature to 400°C for 15 min, withdraw the crucible, allow to cool, dissolve in 1.5 ml of concentrated nitric acid, wash the crucible carefully and collect the solution and the wash water within a 125 ml separation funnel; (3) add a drop of methyl orange solution and neutralize when the intermediate color of the indicator is reached with a 4 N ammonium hydroxide solution, or a 4 N nitric acid solution, or dilute solutions of either; (4) extract the vanadium agitating for 3 min with three successive amounts of 5 ml of 0.5% 8-hydroxiquinoline solution in chloroform, allowing the chloroform layers to pass into another separator funnel containing 30 ml of ammoniacal buffer solution; agitate the chloroform extract an ammoniacal solution for 5 min, allowing the chloroform layers to pass into another separator funnel containing 20 ml of ammoniacal buffer solution, and agitate again for 5 min; dispose of the chloroform layer and add the two extracted layers, washing with 5 ml of alcohol free chloroform twice; (5) add one drop of methyl orange solution in the aqueous phase, precipitated and washed, and neutralize upon reaching the intermediate color with a solution of nitric acid; before the final adjustment of the pH, cork the separator funnel, invert it, collecting all the material that adheres to its wall; this adjustment must be as precise as possible, using dilute

solutions of [illegible]ne acid and of the base, nitric acid, and ammonium hydroxide; (6) add 2 ml of potassium phtalate buffer solution and re-extract the vanadium by agitating for 5 min with 5.0 ml of 0.1% 8-hydroxiquinoline solution in chloroform; (7) let rest for 10 min for the separation of the liquid layers and then collect the chloroform layer in a test tube, and pass it from this test tube to the read-out sterilizing tray of the spectrophotometer; (8) zero adjust with a 0.1% solution of 8-hydroxiquinoline in chloroform and read out the unknown solution on the 550 μ wavelength; (9) the curve is plotted with the various solutions of vanadium subjected to the entire analytical process; on the model B Beckman spectrophotometer, the factor thus found was 72.8189, obtained by dividing the sum of the concentrations by the sum of the readouts; (10) the result is given by the following formula:

$$\text{Result in } \mu g/100 \text{ ml} = \frac{72.8189 \times 100 \times \text{readout}}{\text{serum volume}}$$

Results and Discussion

Table 1 summarizes the major characteristic results found in patients, observing the progressive evolution of the aspect of talipes and pes cavus, the garter atrophy, and the Aran-Duchenne type hand. An electromyographic examination yields evidence in all four cases of progressive deterioration of the anterior tibial muscle, the common extensor of the feet and of the interosseous tissues of the hands.

Although we do not know how to interpret this, the finding of monocytopenia is interesting in the case of amyotrophy under consideration.

The high concentrations of copper and ceruloplasmina in the first two cases and which were less so in the other two patients may reveal an intensive reactional process in the initial cases of the disease which continues decreasing as the disease progresses. These results may perhaps suggest an infectious process as

the trigger factor.

A study of the conduct of the vanadium which suggested the title of the present study deserves a more extensive comment. The values which we accepted as "normal" were determined for four individuals who had no alteration of the nervous system or of the muscles (two cases of carcinoma of the pre-invasion of the uterus type and two healthy individuals).

Little is known of the metabolism of vanadium; however, some facts are already known: Neuberg and Kobel (1926) [27] observed that vanadium accelerates hydrolysis of the hexose diphosphoric acid by yeast extracts; Rygh (1949a, b) [32, 33] observed that a diet free of vanadium reduces by 50% the contents of phosphorus and calcium of the bones in rats; the studies of Bernheim and Bernheim (1938, 1939) [1,2] show that the oxidations which occur in tissues may be accelerated by the presence of vanadium; Snyder and Cornatzer (1958) [37] verified that the administration of vanadium sulfate to rats induces the incorporation of methionine in hepatic proteins and their influence on the metabolism of sulfur appears to be one of the more important aspects of the physiological action of vanadium as may be inferred from the studies of Mountain et al. (1953, 1955, 1963) [24, 25, 26].

Thought has been given to the existence of an inverse relationship between vanadium and copper in the liver (Mountain, 1963) [24] and this is one of the reasons why we added in Table 1 the results found for copper and ceruloplasmine.

Recent studies suggest that vanadium inhibits the synthesis coenzyme A and produces the disappearance of thioctic acid in tissues when found in toxic concentrations (Mascitelli-Coriandoli and Citterio, 1959 a, b) [20, 21] and in these cases, the mechanism of the metal's effect appears to be interfering in the metabolism of sulfhydrillic substances (Mountain et al., 1955; Snyder and Cornatzer, 1958) [26, 27].

Lipides suffer the influence of vanadium, since the administration of this product yields a reduction of the contents of cholesterol and phospholipides of the liver in rabbits, without altering the blood level, by means of a probable mechanism of intervention in the synthesis and the mobilization of cholesterol (Curran and Costello, 1956), [9]; the administration of vanadium causes a highly significant reduction of the cholesterol content in the aorta (Faulkner Hudson, 1964) [13]; the results found by Curran (1954) [7] in experiments conducted on rats give evidence of a reduction of the triglycerides of the liver and those conducted by Curran et al. (1958) [8] show that during the administration of vanadium, the content of blood cholesterol increases in human beings.

The metal causes an increase of the effect of the oxidase monoamine enzyme, in vitro, (Perry et al., 1955) [30] and in the absence of this enzyme, it oxidizes the serotinin as well as the related amines (Martin et al., 1960) [19].

It is possible that vanadium plays an important role in the etiopatogeny of arterial hypertension, in general, as may be inferred from Schroeder's studies (1957, 1960) [34, 35].

The intravenous administration of vanadium gluconate in rabbits produces a significant reticulocytosis and a slow development of polycythemia (Trummert and Beohm, 1957) [39].

Administering vanadium salts to animals, a large part of this material is retained by the bones (Proescher et al., 1917) [31], and in rats, Scott et al. (1951) [36] administered sodium hexavanadate containing ^{48}V and at the end of four days, the bones contained 9.9%, the liver 6.2%, muscles 5.0%, kidneys 4.4%, and blood 1.1%; after 64 days, 10% of the vanadium injected still remained in the body, and among these, 4.6% in the skeleton. Rats poisoned by inhaling carnotite dust showed a major concentration of vanadium in their lungs (Wilson et al., 1953) [42].

Comparing the values encountered in patients under consideration with those of the blood serum of individuals not showing alterations of their nervous system or of the muscles, it is noted that in the charcot-Marie amyotrophy (Table 1) the vanadium levels are much lower in the blood and this reduction is proportional to the gravity of the illness.

These results suggest two explanatory hypotheses: (1) the organism is deficient in vanadium, and (2) the vanadium is deposited in the tissues.

The second hypothesis appears to be more probable, for due to known factors which we discussed earlier, the vanadium is related to the metabolism of the nervous system as a result of its effect on the lipides and sulfur compounds and also in relation to the coenzyme A and the thioctic acid and the oxidase monoamine enzyme. If we search for analog factors, we will find the Wilson disease or hepatolenticular degeneration in which the blood serum has a low copper level and no ceruplasmine is found in the blood. In this disease, the copper deposits on the nuclei of the base and in the liver and also in other organs.

If the analogy is true, it is possible that there is a protein containing vanadium (similar to ceruplasmine) and in the case of the disease under consideration, it is reduced or absent, resulting in the deposition of vanadium in the structures which are damaged in this disease.

From the practical viewpoint, if the pathogeny is per chance due to accumulation of vanadium, it is then necessary to find methods for overcoming it in order to eliminate it from the organism in a form similar to the one used in the treatment of Wilson's disease with penicillamine.

Some investigations aimed at combating the toxic action of vanadium have already been completed: Mitchell (1953) [22] verified that the use of the calcium salt of ethyline diamine tetracetic acid protects mice against lethal doses of vanadium; another effective elimination agent for vanadium is catechol disulfanate

sodium (Braun and Lusky, 1959) [5a]; likewise, vitamin C in large doses may be effective in preventing the death of animals due to vanadium salts (Mitchell and Floyd, 1954) [23].

It is possible that more detailed investigations into the biochemistry of vanadium may contribute to the clarification of the findings in the amiotrophy of charcot-Marie, the object of the present study, as well as in diseases related to the metabolism of compounds containing sulfur.

Table 1. Vanadium in the amyotrophy of charcot-Marie (4 cases in the same family)

Patients	Age (years)	Hollow equine and stiff foot	Atrophy garter	Aran-Duchenne type hand	Monocytes in the blood (%)	Vanadium (µg/100 ml)	Copper (µg/100 ml)	Ceruloplasmine (mg/100 ml)
1st case	12	+	+	+	1	43	200	50.5
2nd case	14	++	+	++	1	40	205	53.4
3rd case	23	++++	++++	++++	4	22	180	41.3
4th case	25	++++	++++	++++	0	18	150	36.6
Normal						80 (70-89)	106 [11] (96-120)	34.0 [10] (28-38)

Note: The normal values of copper and ceruplasmine correspond to references [10] and [11].

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